Neuroscience 158 (2009) 922–931

HIGH FORCE REACHING TASK INDUCES WIDESPREAD INFLAMMATION, INCREASED SPINAL CORD NEUROCHEMICALS AND NEUROPATHIC PAIN

M. B. ELLIOTT,[a,b] A. E. BARR,[b] B. D. CLARK,[c] M. AMIN,[a] S. AMIN[a] AND M. F. BARBE[a,d]*

[a]*Department of Physical Therapy, Temple University, 3307 North Broad Street, Philadelphia, PA 19140, USA*

[b]*Department of Physical Therapy, Thomas Jefferson University, 130 South 9th Street, Philadelphia, PA 19107-5233, USA*

[c]*Department of Neurobiology and Anatomy, Drexel University College of Medicine, 2900 Queen Lane, Philadelphia, PA 19129, USA*

[d]*Department of Anatomy and Cell Biology, Temple University Medical School, 3400 North Broad Street, Philadelphia, PA 19140, USA*

Abstract—Repetitive strain injuries (RSI), which include several musculoskeletal disorders and nerve compression injuries, are associated with performance of repetitive and forceful tasks. In this study, we examined in young, adult Sprague-Dawley rats, the effects of performing a voluntary, moderate repetition, high force (MRHF; nine reaches/min; 60% maximum pulling force) task for 12 weeks on motor behavior and nerve function, inflammatory responses in forearm musculoskeletal and nerve tissues and serum, and neurochemical immunoexpression in cervical spinal cord dorsal horns. We observed no change in reach rate, but reduced voluntary participation and grip strength in week 12, and increased cutaneous sensitivity in weeks 6 and 12, the latter indicative of mechanical allodynia. Nerve conduction velocity (NCV) decreased 15% in the median nerve in week 12, indicative of low-grade nerve compression. ED-1 cells increased in distal radius and ulna in week 12, and in the median nerve and forearm muscles and tendons in weeks 6 and 12. Cytokines IL-1α, IL-1β, TNF-α, and IL-10 increased in distal forearm bones in week 12, while IL-6 increased in tendon in week 12. However, serum analysis revealed only increased TNF-α in week 6 and macrophage inflammatory protein 3a (MIP3a) in weeks 6 and 12. Lastly, Substance P and neurokinin-1 were both increased in weeks 6 and 12 in the dorsal horns of cervical spinal cord segments. These results show that a high force, but moderate repetition task, induced declines in motor and nerve function as well as peripheral and systemic inflammatory responses (albeit the latter was mild). The peripheral inflammatory responses were associated with signs of central sensitization (mechanical allodynia and increased neurochemicals in spinal cord dorsal horns).

Key words: spinal cord, macrophages, cytokines, musculoskeletal disorder, nerve injury, repetitive strain injury.

Repetitive strain injuries (RSIs) are associated with several common pain conditions including back pain, arthritis and musculoskeletal pain. The estimated cost of these disorders is high ($61.2 billion annually) when considering the cost of health care to treat these disorders and lost productivity (Stewart et al., 2003). Epidemiological evidence suggests that nerve compression injury of the upper extremity is associated with the performance of repetitive and forceful tasks (see Barr et al., 2004 for review). In fact, repetitive motion such as typing and repeated grasping was the exposure that resulted in the longest absences from work in 2005 and 2006 (BLS, 2007). One of the most common compressive neuropathies affects the median nerve and is clinically referred to as carpel tunnel syndrome. In 2005 and 2006, carpel tunnel syndrome was listed as one of the most severe of all disabling injuries and illnesses having the highest median days away from work (BLS, 2006, 2007). Patients with this syndrome have symptoms such as pain in the hand and wrist that may travel into the forearm, elbow, and shoulder, as well as paresthesias, numbness and weakness.

Investigations of peripheral nerve compressive injury induced by repetitive motion report reduced nerve conduction velocity (NCV), decreased grip strength, performance declines, inflammation and fibrosis as a result of task performance (Clark et al., 2003, 2004; Sommerich et al., 2007). There are also laboratories studying nerve compression using invasive, surgically induced injuries to the sciatic nerve (Winkelstein et al., 2001a; Gupta and Steward, 2003; Pitcher and Henry, 2004; Hu et al., 2007) and median nerve (Diao et al., 2005). These latter studies have found tactile allodynia, reduced NCV, endoneurial macrophage infiltration, spinal cord neuroplasticity and augmented neuronal excitation, as well as spinal cord inflammatory responses after peripheral nerve injury. In addition to the effects on peripheral nerve, several laboratories, including our own, have documented the effects of repetitive motion on musculoskeletal tissues, including inflammatory cell infiltrates, tendinopathy, degenerative changes and tissue necrosis (Soslowsky et al., 1996; Willems and Stauber, 1999; Barbe et al., 2003; Barr et al., 2003; Geronilla et al., 2003; Diao et al., 2005; Nakama et al., 2005; Perry et al., 2005; Baker et al., 2007; Sommerich et al., 2007).

*Correspondence to: M. F. Barbe, Department of Physical Therapy, Temple University, 3307 North Broad Street, Philadelphia, PA 19140, USA. Tel: +1-215-707-4896; fax: +1-215-707-7500.
E-mail addresses: mary.barbe@temple.edu (M. F. Barbe), melanie.dion@temple.edu, melanie.elliott@jefferson.edu (M. B. Elliott), ann.barr@jefferson.edu (A. E. Barr), brian.clark@drexelmed.edu (B. D. Clark), mamta@temple.edu (M. Amin), shreya1@temple.edu (S. Amin).

Abbreviations: ED1+, ED1-positive activated macrophages; HRNF, high repetition, negligible force; H&E, hematoxylin and eosin; IFNγ, interferon gamma; IL-1, interleukin 1; LRNF, low repetition, negligible force; MIP2, macrophage inflammatory protein 2; MIP3a, macrophage inflammatory protein 3a; MRHF, moderate repetition, high force; NC, normal control; NCV, nerve conduction velocity; RSI, repetitive strain injury; TC, trained control; TNF, tumor necrosis factor.

0306-4522/09 © 2009 IBRO. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuroscience.2008.10.050

Our laboratory has developed a rat model of RSI in which rats perform a voluntary, repetitive, upper extremity task. We have examined the effects of a high repetition, negligible force (HRNF) task and found that performance of this task for 8–12 weeks induces motor declines, local and systemic inflammatory responses in forearm nerve and musculoskeletal tissues, fibrotic compression of the median nerve and a modest, yet significant, 9% decline in NCV (Barbe et al., 2003, 2008; Clark et al., 2003; Barr et al., 2004; Al-Shatti et al., 2005; Elliott et al., 2008). The inflammatory response began in week 3, peaked between 5 and 8 weeks, and included increased macrophages and proinflammatory cytokines in the involved nerves, muscles, tendons, bones, and synovial tissues (Barbe et al., 2003, 2008; Barr et al., 2003; Al-Shatti et al., 2005). We also observed increased levels of pro-inflammatory cytokines and chemokines in serum (Barbe et al., 2003, 2008). Investigation of an HRHF task by our laboratory found motor deficits, cutaneous hyposensitivity, a 17% decline in NCV, and inflammatory and fibrotic changes in the median nerve by 12 weeks (Clark et al., 2004). We recently examined the performance of a low repetition, negligible force (LRNF) task and found that performance of this low demand task induced mild tissue inflammation in nerve and bone, increased neurochemicals (substance P and neurokinin-1) in spinal cord dorsal horns and declines in fine motor control but no declines in gross motor function (Elliott et al., 2008).

Our finding of a neurochemical response in the spinal cord after performance of a low demand task prompted us to explore the relationship between pain behaviors and spinal cord neurochemical changes in animals with higher levels of task-induced inflammation, since Woolf and Salter (2000) proposed a mechanism of inflammation-induced sensitization that drives plasticity of sensory afferents and spinal cord neurons. Only recently has evidence for involvement of the spinal cord in the pathology associated with peripheral nerve compression injuries and pain been provided (Pitcher and Henry, 2004; Hubbard and Winkelstein, 2005; Rothman et al., 2005; Chao et al., 2008). The purpose of the present study was to examine whether pain behaviors and declines in motor function are associated temporally with peripheral inflammation and spinal cord neuroplasticity in rats performing a moderate repetition, high force (MRHF) task, a task exposure that we have not yet explored. Specifically, mechanical hypersensitivity, grip strength and other motor behaviors, NCV, peripheral tissue (forelimb muscle, tendon, distal bone, and nerve) and systemic inflammation, and spinal cord neurochemicals were examined.

EXPERIMENTAL PROCEDURES

Subjects

Sprague–Dawley rats (adult females, 3.5 months of age at onset of experiments) were obtained from ACE, Boyertown, PA, USA. The rats were housed in the Central Animal Facility on the Health Sciences Campus at Temple University. Animal care and use were in compliance with the provisions of federal regulations and the NIH "Guide for the Care and Use of Laboratory Animals" monitored by the University Animal Care and Use Committee (IACUC). Rats were allowed free access to water. The lowest number of animals were used that were needed to reach statistical power of 80%. Any possible animal discomfort was kept to a minimum in accordance with NIH and IACUC guidelines. Experimental and trained control (TC) rats learned to reach for the food during an initial 7–10 day shaping period in which access to food was restricted in order to motivate them to learn the task. Some animals may have undergone a short period (no more than 7 days) of weight reduction to 80% of the weights of the age-matched control group that did not undergo food restriction. Once the animals learned the task, they rapidly gained weight and were maintained at ±5% of age-matched control rats' weights. Rats were weighed twice weekly and food adjusted accordingly.

Repetitive movement task

Fifty-one rats were randomized into one of three groups: moderate repetition, high force group (MRHF; $n=20$), a normal control group (NC; $n=21$) and a trained control group (TC; $n=10$). The MRHF rats and the TC rats (the latter undergoing the initial training (shaping) only), learned to reach for a food pellet (45 mg purified formula pellet, Bioserve, Frenchtown, NJ, USA) for a 2–3 week shaping period, as described earlier in Barbe et al., 2003; Elliott et al., 2008; Clark et al., 2003, 2004. Rats were allowed to use their preferred limb to reach, hereafter referred to as the "preferred limb."

The task rats then performed an MRHF reaching and grasping task for a food pellet for 2 h/day, 3 days/week for 6 ($n=4$) or 12 ($n=16$) weeks using operant test chambers for rodents (Med. Associates, Albans, VT, USA) and a custom designed handle pulling apparatus (Custom Medical Equipment, Glendora, NJ, USA), as described in Clark et al., 2004. The daily task was divided into 4, 0.5-h training sessions separated by 1.5 h. The defined target rate was four reaches/min, although the rats reached above this target maintaining an average, actual reach rate of 9.4 reaches/min. Pull force for this study was set at 60±5% of maximum voluntary pulling force. Maximum pulling force was determined on the last day of the initial training period during a 5 min session in which the force criterion for a food reward was gradually increased. Animals were observed carefully for their maximum force generating ability during this 5 min session, and maximum voluntary pulling force of the force lever handle was selected as the highest force resulting in a successful reach (i.e. food pellet reward) that could be repeated three times. Force threshold criteria established a window in which force was maintained for at least 50 ms for animals to obtain a food reward.

Sensorimotor behavioral testing

The effects of the task on motor performance were evaluated in experimental rats ($n=20$), NCs ($n=21$) and TCs ($n=10$) by examining task duration (minutes/day), reach rate (reaches/min), mechanical cutaneous sensation of the forepaws using the Von Frey test (Chaplan et al., 1994) and median grip strength as described previously (Barbe et al., 2003; Clark et al., 2003, 2004). Mechanical cutaneous sensation and grip strength are reported as mean maximum force in grams and standard error of the mean (S.E.M.) for preferred limbs.

NCV

In order to test focal slowing of conduction (Kimura, 1979; Walters and Murray, 2001), NCV was determined for the segment of the median nerve that passes beneath the transcarpal ligament. NCV was measured in terminal surgical experiments in 10 NC rats (20 limbs) and in eight rats that had performed the MRLF task for 12 weeks. The method for measurement of NCV of the median nerve in rats is as described previously in Clark et al. (2003, 2004). All